

Engineering human organoid development *ex vivo*—challenges and opportunities

Oriane B. Matthys^{1,2}, Ana C. Silva¹ and Todd C. McDevitt^{1,3}

Abstract

The rapid progress of organoid technologies is attributable to the application of developmental biology principles, but organoid methods need further refinement provided by engineering approaches. However, we must first begin with common consensus on the critical features that distinguish organoids from simpler microtissues that lack the cellular complexity, structure, and function that organoids can achieve. Furthermore, current abilities to derive organoids from stem cells or multipotent progenitors favor certain germ lineages and tissue types more so than others, although the full reasons for this imbalance have yet to be determined. Technical challenges remain to identify the critical starting parameters for organoid reproducibility, systematically manipulate the proportions of differentiated cells from progenitors, and comprehensively characterize cell phenotypes spatially using advanced transcriptomic and 3D imaging methods. Advances in these regards will undoubtedly improve the robustness and predictability of existing organoids and permit the creation of organoids that have yet to be described.

Addresses

¹ Gladstone Institutes, San Francisco, CA, USA

² UC Berkeley-UC San Francisco Graduate Program in Bioengineering, San Francisco, CA, USA

³ Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, USA

Corresponding author: McDevitt, Todd C (todd.mcdevitt@gladstone.ucsf.edu)

Current Opinion in Biomedical Engineering 2020, 13:160–167

This review comes from a themed issue on **Futures of Biomedical Engineering: Bioengineering of Organoids and Tissue Development**

Edited by **George Truskey** and **Jianping Fu**

<https://doi.org/10.1016/j.cobme.2020.03.001>

2468-4511/© 2020 Elsevier Inc. All rights reserved.

Keywords

Organoids, Tissue engineering, Stem cells, Morphogenesis.

Introduction

Embryos have traditionally served as the primary model to study tissue specification and developmental processes, but ethical considerations limit the ability to interrogate human morphogenic events in a similar manner. Thus, the need to create tractable *in vitro*

platforms to directly examine critical aspects of human development has led to the pursuit of organoids and their ability to recapitulate embryonic morphogenesis. The rapid emergence and widespread use of diverse organoid models has been achieved by combining developmental biology principles with more robust engineering technologies to significantly improve our understanding of complex human morphogenic phenomena.

Since the earliest days of pluripotent stem cell (PSC) biology, 3D culture methods, such as hanging drop, have been used to assess differentiation potential. Embryoid bodies (EBs), 3D spheroidal constructs of PSCs that spontaneously self-assemble and differentiate into multiple germ lineages in parallel, were the first PSC model intended to emulate organotypic morphogenesis [1–3]. However, EB differentiation is notoriously heterogeneous and inconsistent between batches, and individual spheroids exhibit highly variable proportions of differentiated cells from different germ lineages. The uncontrolled cellular heterogeneity was largely due to the complexity and inconsistency of serum composition—the primary inducer of EB differentiation. However, as developmental signaling pathways and cell fate specification became better understood, more defined cocktails of morphogens and/or chemical compounds replaced the prevalent use of serum, which facilitated directed differentiation to specific germ lineages and the creation of organoids that recapitulate tissue-specific multicellular organization and functional properties. In addition to tissue-specific organoids, *ex vivo* models of specific stages of embryonic development have emerged by mimicking developmental signals. For example, ‘embryoids’ or ‘gastruloids’ recapitulate the earliest cell specification and symmetry breaking events before the emergence of distinct tissues [4–6], thereby vastly improving on the stochastic nature of EB cellularity and architectural arrangement.

The ability to generate a diverse array of organoid models from human PSCs in conjunction with current gene editing technologies has afforded many new opportunities for the development of organoids as biological tools to study tissue homeostasis and disease. For instance, introducing oncogenic mutations into cerebral organoids via CRISPR-Cas9 gene editing recapitulates brain tumorigenesis [7], whereas the effects of infectious disease outbreaks can be directly examined by infecting developing forebrain organoids with Zika virus,

resulting in reduced neuronal cell volume reminiscent of microcephaly [8] and thus offering a screening platform to test potential antiviral drugs [9]. Organoid models also enable drug discovery for hereditary diseases, such as lung [10] and intestine [11] organoids derived from patients with cystic fibrosis that were used to identify compounds capable of restoring function. Organoids can not only advance the study of host–pathogen interactions [12,13], but also enable biomanufacturing of human pathogens, such as noroviruses, that can only be cultivated in the appropriate physiologic environment provided by human enteroid models [14]. Recent studies also demonstrate unique applications of organoids, such as snake venom gland organoids that produce functionally active toxins, which can facilitate the development of various antidotes [15]. However, organoid technologies still face several challenges, such as the inherent variability between individual organoids, experiments, and cell lines, and limited examples of mesoderm-derived organoids relative to current ectoderm and endoderm models. Here, we survey recent progress in the field of organoid engineering, by first discussing the necessary criteria that define 3D tissue constructs as organoids, evaluating the current landscape of organoid development, and proposing solutions for current limitations.

A consensus definition for ‘organoids’

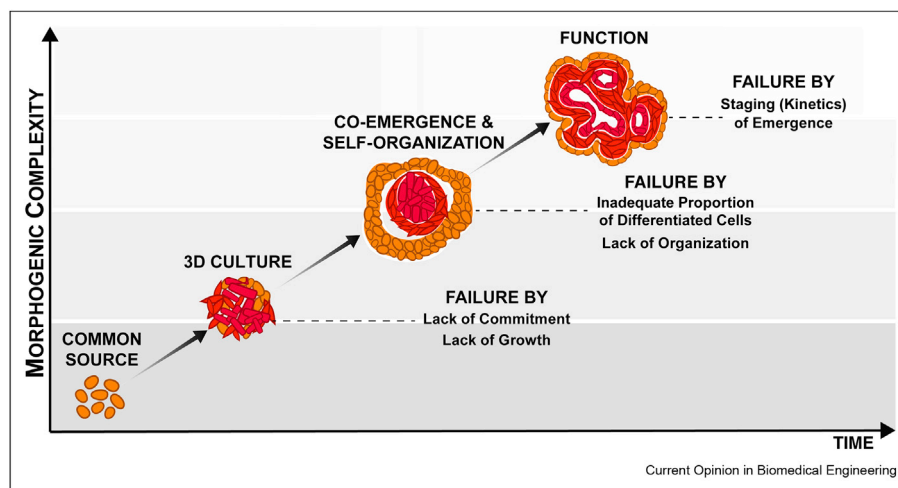
Analogous to the functional criteria used to define ‘bona fide’ stem cells, similar requirements must be met for an *in vitro* tissue construct to be properly classified as a true ‘organoid’: 1) a multi-lineage stem/progenitor cell source, 2) 3D multicellular structure, 3) co-emergence and self-organization of multiple distinct cell types,

and 4) functional properties resembling the corresponding native tissue (Figure 1).

PSCs and multipotent progenitors are innately capable of generating organoids because of their ability to give rise to multiple types of differentiated progeny. PSCs are advantageous because they can serve as a single isogenic source of various multipotent progenitor populations by first directing their specification to distinct germ lineages that yield tissue-specific progenitor(s). In addition, the relative ease of genomic editing of PSCs enables monitoring the emergence of specific cell types with transgenic fluorescent protein expression, as well as interrogation of the impacts of specific genes on tissue development, for example, by gene inhibition (CRISPRi) or activation (CRISPRa). Alternatively, multipotent progenitors obtained from tissue sources (often via biopsy) have more restricted differentiation capacity and do not exist for all tissues. Nevertheless, sampling multiple progenitors from different individuals or patient populations enables disease modeling for conditions that have not yet been described for PSCs. Although intestine [16–18], liver [19–21], and lung [10,22] organoids have been successfully generated from both PSC- and tissue-derived cells, many other models have only been achieved with one and not the other, such as several different brain organoids [8,23]. The complementary use of different progenitor cell sources affords a wealth of different types of organoids that can be adapted for particular tissue or disease modeling applications.

As a first step towards recapitulating native tissue structure, stem/progenitor cells are typically cultured in

Figure 1



Critical features of organoid generation. 1) Common stem or progenitor cell source; 2) three-dimensional tissue culture; 3) co-emergence and self-organization of multiple tissue-specific cell types; and 4) functional attributes that mimic target tissue.

a 3D format. Most often, cells are aggregated to promote intimate cell contact in a 3D multicellular arrangement. This mimicry of cell condensation is most often achieved through a combination of spontaneous aggregation [17,24] and suspension culture methods [8,19,23]. Entrapment of pluri- or multipotent progenitors, either as single cells or spheroids, in hydrogels can enable longitudinal analysis of individual organoids as well as provide further specification cues. Matrigel has been used extensively as an encapsulation material and/or supplement in cell culture media during early stages of organoid differentiation, providing a complex microenvironment that instructs cell specification and patterning events, such as the establishment of apical/basal polarity, neuroepithelial buds, cortical plate formation, retinal primordium morphology, and posterior gut tube epithelial patterning [18,25,26]. Synthetic hydrogels are less common in established organoid protocols, but are increasingly being examined as support scaffolds for survival and maintenance of intestine and lung organoids [27,28]. Synthetic polymer microfilaments have also been exploited to induce brain organoid elongation, improve neuroepithelial polarization, and direct differentiation to specific brain regions [25]. Both natural and synthetic biomaterials have been observed as essential for organoid morphogenic and patterning complexity, yet there is still a need to dissect the mechanisms behind the biomaterial impacts, for example, determining whether the specific contributions are a result of mechanical properties, chemical composition, material shape, or most likely, a combination of these factors.

The most critical phase transition of organoid generation from any progenitor source is the co-emergence of multiple cell types that occurs through the parallel specification of different cell types. The emergence of distinct phenotypes is not an entirely random event, but rather, resembles *in vivo* morphogenesis. The proper balance, spatially as well as temporally, between different cell types is a critical determinant of organoid formation that seems to be self-regulated by the multicellular entity without exogenous intervention. The necessary result of organization is that cells are spatially primed (via physical or paracrine interactions) to promote proper tissue structure and function. However, not all multicellular organization events will support successful organoid generation; instead, tissues may fail to self-organize altogether—remaining a collective mass of heterogeneous cells that will not yield functional outputs—or, organization events may occur in a manner that does not produce the structure needed for subsequent tissue growth, specification, or function [23]. Cell organization events can occur over the course of hours to days and can be as simple as the formation of cystic-like liver or fallopian tube spheroids [19,29], or as complex as stratified layers of cerebral organoids [23,25]. Fallopian tube and liver organoids start as progenitor cells embedded in Matrigel that expand into

cystic-like spheres that are then cultured in differentiation media promoting the specification and maturation into distinct tissue-specific cell types [19,29]. Organoids with stratified architecture are more prone to organizational failure because of the complexity of the mechanisms underlying the cascade of events that precedes organoid formation. Brain organoids, for example, can fail at multiple stages, from neuroectoderm induction to bud formation or erroneous cystic formation [23].














Tissue-specific functional attributes manifest once the developing organoid has specified multiple cell types and self-organized into a permissive arrangement. At least one functional property specific to the native target tissue must be demonstrated to declare an *in vitro* tissue ‘an organoid.’ Proper organoid function can be assessed in multiple ways, including electrophysiological measures [30], detection of hormone and enzyme secretion [18,29,31–33], and physiological response to chemical compounds or mechanical stress [29,31–34]. For electrically excitable tissues, such as those composed of neurons or cardiomyocytes, the assessment of calcium handling and action potential properties is critical to describe cellular maturation and subtype specification. Other organoid models that exhibit physiological metabolic functions (i.e. gut, liver, kidney) can be evaluated by detecting secreted molecules or metabolites or by characterizing their response to chemical stimuli [33]. These methods of functional quantification are nondestructive and therefore can be used to longitudinally assess organoid development. Lastly, for organoids that do not exhibit measurable electrical or chemical activity, such as skin organoids, function can be evaluated by the appearance of hallmark architectural features, such as the development of hair follicles [35,36].

Altogether, these four critical features (at a minimum) should be demonstrated before the assertion is made that a 3D tissue construct is truly deemed worthy of being described as an ‘organoid.’ Thus, conservative use of the term ‘organoid’ should be followed by researchers for the purposes of clarity and accuracy.

The contrasting landscape of organoid development

The diversity of organoid models has rapidly accelerated because of advances in fundamental principles of developmental biology and engineering stem cell technologies. However, many bona fide examples of organoids that fulfill the aforementioned criteria still remain to be created. For instance, the current landscape of the field reveals a propensity for the generation of ectoderm- and endoderm-derived organoids, whereas many highly desirable mesoderm-derived organoid models are completely absent or lacking in terms of cellular complexity, higher order structure, and functional properties (Figure 2).

Figure 2

		Source	Type	References
ECTODERM	 Cerebral	PSC	stratified	15, 16, 18, 31
	 Retinal	PSC	stratified cystic	19, 43
	 Skin	PSC	stratified	28, 29
	 Salivary Gland	biopsy	branching	34
	 Mammary Gland	biopsy	branching	32, 33
MESODERM	 Kidney	PSC	branching	27, 42
	 Fallopian tube Endometrial	biopsy	cystic	22, 24, 25
ENDODERM	 Intestinal	PSC biopsy	branching	7 - 9, 20
	 Gastric	PSC biopsy	cystic	38
	 Liver	PSC biopsy	cystic	10-12, 17, 26
	 Lung	PSC biopsy	cystic	13, 14, 21
	 Thyroid	PSC	cystic	36
	 Pancreas	biopsy	branching cystic	35

Current Opinion in Biomedical Engineering

Survey of organoid landscape by developmental origin. Tissue type, progenitor cell source, morphogenic structure, and relevant references.

During development, specification of germ lineages occurs first with the bifurcation of ectoderm from mesendoderm followed by the division of mesendoderm into endoderm and mesoderm [37]. Ectoderm,

positioned as the outer layer in the embryo, gives rise to the nervous system and epidermis; thus, ectoderm-derived organoids range from neural subtypes (non-specific [23] to regional-specific [8,38] models; optic

cup [26]), which are the most widely adopted organoid models, to a few non-neural ectoderm models, such as skin and inner ear hair follicle organoids [35,36] and mammary and salivary gland [39–41] organoids. Ectoderm-derived organoids tend to develop radially stratified organizational structures with the exception of the gland organoids that undergo branching/budding phenomena.

Endoderm-derived organoids also commonly exhibit cystic and branching/budding structures. Endoderm-derived organoids (pancreas [42], liver [19,21], lung [10,22], thyroid [43]) start by specifying definitive endoderm and are largely generated from multipotent tissue-derived cells, as most tissues of endoderm lineage typically retain a regenerative capacity attributable to the presence of resident tissue self-renewing progenitors. Furthermore, many of the established endoderm-derived organoids require a mesenchyme/stromal support population for tissue development and higher order structural specification, which may be ascribed to the shared ancestry of the initial embryonic mesendoderm lineage. For example, mesenchyme factors FGF10 and laminin are necessary for pancreatic progenitor maintenance and expansion as well as for polarity induction, respectively. Furthermore, liver bud organoids require mesenchymal support (either from primary bone marrow-derived mesenchymal stromal cells [24] or PSC-derived mesenchyme [20]) for spontaneous condensation to occur [44].

Although a few mesoderm-derived organoid models exist, including kidney [34], fallopian tube [29], and endometrium [31,32], many desirable mesoderm tissues that meet the aforementioned organoid criteria (organization of multiple cell types arising from a common progenitor population) are notably lacking in the field. Those mesoderm organoids that do exist share common characteristics with several endoderm and ectoderm tissues, such as tissue-resident epithelial source that gives rise to fallopian tube and endometrial organoids [29,31,32]. Furthermore, the generation of fallopian tube organoids mimics gastrointestinal organoid culture methods to preserve stemness and expand fallopian epithelial cells, even using gastric organoid medium [29,45], whereas endometrial organoid creation uses multiple shared factors with liver organoids to promote self-renewal and expansion [21,31,32]. Therefore, successful generation of mesoderm-derived organoids may require significantly different types of approaches than those that have been largely successful for epithelial-based tissue models. For example, the lack of other mesoderm organoids could be due to the diverse origins of mesoderm-derived tissues, which originate from progenitor cells that migrate long distances from distinct developmental regions to converge during tissue development, such as primary and secondary heart field and neural crest cells [46]. The absence of

mesoderm-derived tissue models, such as the heart, may also be due to the lack of a native postnatal regenerative cell population that can be isolated to create organoids.

The elusive creation of muscle organoids may require more complex cues and multiple progenitor populations. The heart is the first organ to form during embryogenesis through a series of complex organizational events that requires chemical cues and cell migration from surrounding tissues, such as the foregut, neural crest, and proepicardial organ. Therefore, the lack of multiple co-existing germ layers in the same organoid system is perhaps one of the limiting factors for the generation of putative cardiac organoid models. The first muscle-containing organoid was recently reported by differentiating spheroids of bipotent neuromesodermal progenitors (from human PSCs) in 3D to model neuromuscular junctions [47]. Furthermore, the emergent organizational structures observed across many types of organoid models suggest that certain morphogenic phenomena seem to be organizationally more feasible to recreate *in vitro*, such as striated layers [23] and budding/branching [10,17,22,39,40] phenotypes. However, looping and fusion events that contribute to heart chamber development have yet to be achieved *ex vivo*, potentially because these morphogenic processes involve combinations of multiple progenitors that arise differentially in space and time during development.

Current limitations of organoid engineering

Despite the rapid emergence of organoids over the past decade, limitations regarding formation, culture control, and technological analysis continue to hinder the field. However, application of bioengineering tools and techniques to several aspects of organoid manufacturing may improve control of organoid formation, multicellular organization, and multimodal analyses. The precise starting conditions that yield successful organoid generation often remain hard to identify, let alone control. The variable efficiency of successful organoid generation is a major limitation, which is likely due to a number of multicellular parameters, including cell state, ratio of cell types, and cell density, among others. Variability in successful organoid cultures tends to occur more so between batches as opposed to within batches [48,49] analogous to the inherent variability of *in vitro* directed differentiation protocols across different cell lines—something that is also observed when generating organoids [49,50]. Better definition of the critical parameters leading to consistent organoid generation should lead to more consistent outcomes. Early metrics of batch quality (i.e. flow cytometry of multicellular proportions, gene expression ratios of specific cell markers, or sorting for desired morphological features [51]) could allow for identification of starting cell populations that are primed for successful organoid culture.

In addition to variable efficiency of organoid emergence, the inability to control cell specification and organization events directly within organoids suggests that novel cell patterning platforms could enhance the reliability of organoid generation. For example, intrinsically mediated patterning could be achieved by mixing isogenic populations of stem cells with one or more subpopulations having modulated gene expression of specific molecules. This was recently demonstrated by using CRISPRi to knockdown E-cadherin (CDH1) or ROCK1 in a subpopulation of iPSCs, resulting in separation of knockdown cells from naïve iPSCs without the loss of pluripotency [52]. In addition, different spatial patterns of iPSCs predicted by machine learning approaches were created by varying the ratios of cells and temporal kinetics of gene knockdown [53]. This manner of regulating differential gene expression alters the subsequent receptiveness of cells to morphogen signals, which could be used to control cell divergence during organoid development. Similarly, optogenetic control of spatiotemporal transcriptional and intercellular signaling events has been used to manipulate cell fate commitment and stimulate morphogenesis in developmental model organisms [54,55] and could therefore be applied to patterning multicellular PSC organization. Microfluidic systems engineered to control signaling gradients can define extracellular chemical microenvironments that instruct cell patterning events, such as neural tube morphogenesis [56]. Therefore, bioengineering strategies that are informed by developmental biology principles can improve many aspects of organoid creation, such as controlled spatiotemporal organization reminiscent of native patterning.

Technological advances capable of single-cell spatiotemporal resolution are needed to more accurately assess the relationship between organoid specification and organization. Single-cell RNA-sequencing has become a powerful tool to characterize the cellular diversity within organoids and map the temporal trajectory of cell phenotypes and emergence dynamics when sampled frequently enough. However, single-cell RNA-sequencing of dissociated organoids fails to capture spatial information and rare cell populations escape detection unless sufficient sequencing depth is obtained. Spatial transcriptomics integrate histological tissue sectioning with RNA-sequencing to couple spatial information with gene expression [57], but the spatial resolution is not yet at the single-cell level. Furthermore, hundreds of serial sections spanning the entirety of an individual organoid would be required to comprehensively reconstruct 3D spatial structural/phenotypic network information. Advances in 3D microscopy, especially light sheet fluorescence microscopy methods, enable imaging of intact tissues (fixed and live) with increased depth penetration and decreased photobleaching [58,59]. However, although light sheet fluorescence microscopy permits complex

3D spatial mapping with high spatial resolution, it is a relatively low-throughput assay that is limited to a small number of markers at a time and is therefore not amenable to unbiased detection of spatial phenotypes. Ongoing advances in analytical methods for 3D spatial phenotypic mapping should directly benefit organoid research efforts.

Conclusions

The spread of organoid models has yielded novel insights into human development and provided new biological substrates to directly study human homeostasis and disease *ex vivo*. However, rigorous standards should be followed to accurately describe 3D multicellular assemblies of stem/progenitor cells as true organoids and distinguish them from simpler engineered tissue constructs that fail to attain higher order structure and function. Currently, an imbalance in the adoption of organoids is reflected by the relative amount of research that has favored the use of more robust and reproducible systems. Technical advances in methods to control the emergence of different cell types comprising organoids and characterize phenotypic organization and integration will inevitably lead to new and refined organoids that will continue to permeate all aspects of biomedical research.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors would like to acknowledge funding support from the California Institute of Regenerative Medicine (LA1-08015). OBM is a National Science Foundation Graduate Research Fellow (1650113) and ACS is supported by the Gladstone BioFulcrum Heart Failure Research Program. The authors thank the Gladstone art department for scientific illustration support.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1. Hopfl G, Gassmann M, Desbaillets I: **Differentiating embryonic stem cells into embryoid bodies.** *Methods Mol Biol* 2004, **254**: 79–98. <https://doi.org/10.1385/1-59259-741-6:079>.
2. Wiese C, Kania G, Rolletschek A, Blyszczuk P, Wobus AM: **Pluripotency: capacity for in vitro differentiation of undifferentiated embryonic stem cells.** *Methods Mol Biol* 2006, **325**: 181–205. <https://doi.org/10.1385/1-59745-005-7:181>.
3. Carpenedo RL, Sargent CY, McDevitt TC: **Rotary suspension culture enhances the efficiency, yield, and homogeneity of embryoid body differentiation.** *Stem Cell* 2007, **25**:2224–2234. <https://doi.org/10.1634/stemcells.2006-0523>.
4. Simunovic M, Brivanlou AH: **Embryoids, organoids and gastruloids: new approaches to understanding embryogenesis.** *Development* 2017, **144**:976–985. <https://doi.org/10.1242/dev.143529>.
5. van den Brink SC, *et al.*: **Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells.** *Development* 2014, **141**:4231–4242. <https://doi.org/10.1242/dev.113001>.

6. Turner DA, *et al.*: **Anteroposterior polarity and elongation in the absence of extra-embryonic tissues and of spatially localised signalling in gastruloids: mammalian embryonic organoids.** *Development* 2017, **144**:3894–3906. <https://doi.org/10.1242/dev.150391>.
- This study describes a robust model of mammalian embryo (E6.0–7.0) anterior–posterior patterning of caudalized mouse ESCs. The authors demonstrated that contrary to previous reports, gastruloid polarization occurs in the absence of extra-embryonic-like tissues and is dependent on Nodal and Wnt signaling interactions. This gastruloid model suggests that mammalian embryonic symmetry-breaking can occur intrinsically, but the presence of extra-embryonic tissue promotes more robust development.
7. Bian S, *et al.*: **Genetically engineered cerebral organoids model brain tumor formation.** *Nat Methods* 2018, **15**:631–639. <https://doi.org/10.1038/s41592-018-0070-7>.
8. Qian X, *et al.*: **Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure.** *Cell* 2016, **165**:1238–1254. <https://doi.org/10.1016/j.cell.2016.04.032>.
9. Dang J, *et al.*: **Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3.** *Cell Stem Cell* 2016, **19**:258–265. <https://doi.org/10.1016/j.stem.2016.04.014>.
10. Sachs N, *et al.*: **Long-term expanding human airway organoids for disease modeling.** *EMBO J* 2019, **38**. <https://doi.org/10.15252/embj.2018100300>.
11. Dekkers JF, *et al.*: **A functional CFTR assay using primary cystic fibrosis intestinal organoids.** *Nat Med* 2013, **19**:939–945. <https://doi.org/10.1038/nm.3201>.
12. Duque-Correa MA, Maizels RM, Grencis RK, Berriman M: **Organoids - new models for host-helminth interactions.** *Trends Parasitol* 2020, **36**:170–181. <https://doi.org/10.1016/j.pt.2019.10.013>.
13. Dutta D, Clevers H: **Organoid culture systems to study host-pathogen interactions.** *Curr Opin Immunol* 2017, **48**:15–22. <https://doi.org/10.1016/j.coi.2017.07.012>.
14. Ettayebi K, *et al.*: **Replication of human noroviruses in stem cell-derived human enteroids.** *Science* 2016, **353**:1387–1393. <https://doi.org/10.1126/science.aaf5211>.
15. Post Y, *et al.*: **Snake venom gland organoids.** *Cell* 2020, **180**:233–247. <https://doi.org/10.1016/j.cell.2019.11.038>. e221.
16. Sato T, *et al.*: **Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche.** *Nature* 2009, **459**:262–265. <https://doi.org/10.1038/nature07935>.
17. Spence JR, *et al.*: **Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro.** *Nature* 2011, **470**:105–109. <https://doi.org/10.1038/nature09691>.
18. Munera JO, *et al.*: **Differentiation of human pluripotent stem cells into colonic organoids via transient activation of BMP signaling.** *Cell Stem Cell* 2017, **21**:51–64. <https://doi.org/10.1016/j.stem.2017.05.020>. e56.
19. Guan Y, *et al.*: **Human hepatic organoids for the analysis of human genetic diseases.** *JCI Insight* 2017, **2**. <https://doi.org/10.1172/jci.insight.94954>.
20. Takebe T, *et al.*: **Massive and reproducible production of liver buds entirely from human pluripotent stem cells.** *Cell Rep* 2017, **21**:2661–2670. <https://doi.org/10.1016/j.celrep.2017.11.005>.
21. Huch M, *et al.*: **In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration.** *Nature* 2013, **494**:247–250. <https://doi.org/10.1038/nature11826>.
22. Dye BR, *et al.*: **In vitro generation of human pluripotent stem cell derived lung organoids.** *Elife* 2015, **4**. <https://doi.org/10.7554/eLife.05098>.
23. Lancaster MA, Knoblich JA: **Generation of cerebral organoids from human pluripotent stem cells.** *Nat Protoc* 2014, **9**:2329–2340. <https://doi.org/10.1038/nprot.2014.158>.
24. Takebe T, *et al.*: **Vascularized and complex organ buds from diverse tissues via mesenchymal cell-driven condensation.** *Cell Stem Cell* 2015, **16**:556–565. <https://doi.org/10.1016/j.stem.2015.03.004>.
25. Lancaster MA, *et al.*: **Guided self-organization and cortical plate formation in human brain organoids.** *Nat Biotechnol* 2017, **35**:659–666. <https://doi.org/10.1038/nbt.3906>.
26. Nakano T, *et al.*: **Self-formation of optic cups and storable stratified neural retina from human ESCs.** *Cell Stem Cell* 2012, **10**:771–785. <https://doi.org/10.1016/j.stem.2012.05.009>.
27. Cruz-Acuna R, *et al.*: **Synthetic hydrogels for human intestinal organoid generation and colonic wound repair.** *Nat Cell Biol* 2017, **19**:1326–1335. <https://doi.org/10.1038/ncb3632>.
28. Dye BR, *et al.*: **A bioengineered niche promotes in vivo engraftment and maturation of pluripotent stem cell derived human lung organoids.** *Elife* 2016, **5**. <https://doi.org/10.7554/eLife.19732>.
29. Kessler M, *et al.*: **The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids.** *Nat Commun* 2015, **6**:8989. <https://doi.org/10.1038/ncomms9989>.
30. Rigamonti A, *et al.*: **Large-scale production of mature neurons from human pluripotent stem cells in a three-dimensional suspension culture system.** *Stem Cell Rep* 2016, **6**:993–1008. <https://doi.org/10.1016/j.stemcr.2016.05.010>.
31. Boretto M, *et al.*: **Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability.** *Development* 2017, **144**:1775–1786. <https://doi.org/10.1242/dev.148478>.
32. Turco MY, *et al.*: **Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium.** *Nat Cell Biol* 2017, **19**:568–577. <https://doi.org/10.1038/ncb3516>.
33. Huch M, *et al.*: **Long-term culture of genome-stable bipotent stem cells from adult human liver.** *Cell* 2015, **160**:299–312. <https://doi.org/10.1016/j.cell.2014.11.050>.
34. Takasato M, *et al.*: **Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis.** *Nature* 2016, **536**:238. <https://doi.org/10.1038/nature17982>.
35. Koehler KR, *et al.*: **Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells.** *Nat Biotechnol* 2017, **35**:583–589. <https://doi.org/10.1038/nbt.3840>.
36. Lee J, *et al.*: **Hair follicle development in mouse pluripotent stem cell-derived skin organoids.** *Cell Rep* 2018, **22**:242–254. <https://doi.org/10.1016/j.celrep.2017.12.007>.
37. Tada S, *et al.*: **Characterization of mesendoderm: a diverging point of the definitive endoderm and mesoderm in embryonic stem cell differentiation culture.** *Development* 2005, **132**:4363–4374. <https://doi.org/10.1242/dev.02005>.
38. Iefremova V, *et al.*: **An organoid-based model of cortical development identifies non-cell-autonomous defects in Wnt signaling contributing to miller-dieker syndrome.** *Cell Rep* 2017, **19**:50–59. <https://doi.org/10.1016/j.celrep.2017.03.047>.
39. Jamieson PR, *et al.*: **Derivation of a robust mouse mammary organoid system for studying tissue dynamics.** *Development* 2017, **144**:1065–1071. <https://doi.org/10.1242/dev.145045>.
40. Linnemann JR, *et al.*: **Quantification of regenerative potential in primary human mammary epithelial cells.** *Development* 2015, **142**:3239–3251. <https://doi.org/10.1242/dev.123554>.
41. Pringle S, *et al.*: **Human salivary gland stem cells functionally restore radiation damaged salivary glands.** *Stem Cell* 2016, **34**:640–652. <https://doi.org/10.1002/stem.2278>.
42. Loomans CJM, *et al.*: **Expansion of adult human pancreatic tissue yields organoids harboring progenitor cells with endocrine differentiation potential.** *Stem Cell Rep* 2018, **10**:712–724. <https://doi.org/10.1016/j.stemcr.2018.02.005>.

43. Saito Y, *et al.*: **Development of a functional thyroid model based on an organoid culture system.** *Biochem Biophys Res Commun* 2018, **497**:783–789. <https://doi.org/10.1016/j.bbrc.2018.02.154>.
44. Camp JG, *et al.*: **Multilineage communication regulates human liver bud development from pluripotency.** *Nature* 2017, **546**: 533–538. <https://doi.org/10.1038/nature22796>.
 The authors applied single-cell RNA-sequencing to dissect the dynamics of hepatic endoderm differentiation in 3D, with or without the addition of endothelial and mesenchymal stromal cells, in order to understand multilineage cell communication during human liver bud development. This analysis, in combination with receptor-ligand pairing evaluation allowed the identification of VEGF as a crosstalk factor that potentiates endothelial cell mesh formation and hepatoblast differentiation.
45. Barker N, *et al.*: **Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro.** *Cell Stem Cell* 2010, **6**:25–36. <https://doi.org/10.1016/j.stem.2009.11.013>.
46. Gittenberger-de Groot AC, Bartelings MM, Deruiter MC, Poelmann RE: **Basics of cardiac development for the understanding of congenital heart malformations.** *Pediatr Res* 2005, **57**:169–176. <https://doi.org/10.1203/01.PDR.0000148710.69159.61>.
47. Martins J-M, *et al.*: **Self-organizing 3D human trunk neuro-muscular organoids.** *Cell Stem Cell* 2020. <https://doi.org/10.1016/j.stem.2019.12.007>.
 This study describes a neuromuscular organoid model achieved by the aggregation of human iPSC-derived neuromesodermal (NM) progenitors. The NM spheroids demonstrated extensive growth, forming a mature and functional network of neural and muscular tissues. This novel organoid platform was then used to study myasthenia gravis, an autoimmune disorder that affects the integrity of neuromuscular junctions.
48. de Souza N: **Organoid variability examined.** *Nat Methods* 2017, **14**. <https://doi.org/10.1038/nmeth.4362>. 655-655.
49. Subramanian A, *et al.*: **Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation.** *Nat Commun* 2019, **10**:5462. <https://doi.org/10.1038/s41467-019-13382-0>.
 Kidney organoids derived using 2 different protocols and 4 different hPSC lines were analyzed at 4 time points using single-cell RNA-sequencing. The majority of variability among kidney organoids was found to be between hPSC lines as opposed to within batches or sequential batches of the same cell line. Differences between cell lines most strongly impacts the relative proportion of off-target (non-kidney) cell types that arise in the organoids. However, *in vivo* culture of kidney organoids attenuates the emergence of off-target cell populations.
50. Capowski EE, *et al.*: **Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines.** *Development* 2019, **146**. <https://doi.org/10.1242/dev.171686>.
 A multiplexed, image-based classification system was developed to evaluate the consistency and behavior of retinal organoids over time derived from different hPSC lines. These experiments were performed using a hybrid 3D protocol that combined the latest advances in retinal organoid culture techniques described by several laboratories. The effects of epigenetic differences on retinal organoid formation efficiency between hPSC isogenic and non-isogenic lines was examined.
51. Arora N, *et al.*: **A process engineering approach to increase organoid yield.** *Development* 2017, **144**:1128–1136. <https://doi.org/10.1242/dev.142919>.
52. Libby AR, *et al.*: **Spatiotemporal mosaic self-patterning of pluripotent stem cells using CRISPR interference.** *Elife* 2018, **7**. <https://doi.org/10.7554/eLife.36045>.
53. Libby ARG, *et al.*: **Automated design of pluripotent stem cell self-organization.** *Cell Syst* 2019, **9**:483–495. <https://doi.org/10.1016/j.cels.2019.10.008>. e410.
54. Izquierdo E, Quinkler T, De Renzis S: **Guided morphogenesis through optogenetic activation of Rho signalling during early Drosophila embryogenesis.** *Nat Commun* 2018, **9**:2366. <https://doi.org/10.1038/s41467-018-04754-z>.
55. Sako K, *et al.*: **Optogenetic control of nodal signaling reveals a temporal pattern of nodal signaling regulating cell fate specification during gastrulation.** *Cell Rep* 2016, **16**:866–877. <https://doi.org/10.1016/j.celrep.2016.06.036>.
56. Demers CJ, *et al.*: **Development-on-chip: in vitro neural tube patterning with a microfluidic device.** *Development* 2016, **143**: 1884–1892. <https://doi.org/10.1242/dev.126847>.
57. Stahl PL, *et al.*: **Visualization and analysis of gene expression in tissue sections by spatial transcriptomics.** *Science* 2016, **353**:78–82. <https://doi.org/10.1126/science.aaf2403>.
58. Dekkers JF, *et al.*: **High-resolution 3D imaging of fixed and cleared organoids.** *Nat Protoc* 2019, **14**:1756–1771. <https://doi.org/10.1038/s41596-019-0160-8>.
59. Boothe T, *et al.*: **A tunable refractive index matching medium for live imaging cells, tissues and model organisms.** *Elife* 2017, **6**. <https://doi.org/10.7554/eLife.27240>.